

THE METABOLISM OF RHAMNOSE
A NATURALLY OCCURRING METHYL PENTOSE

By ALFRED K. SILBERMAN

A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIRE-
MENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN THE
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REPRINTED FROM
THE JOURNAL OF BIOLOGICAL CHEMISTRY
VOL. 101, No. 3, PAGES 741-751, AUGUST, 1933
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PENTOSE METABOLISM

III. THE RATE OF ABSORPTION OF *l*-RHAMNOSE AND THE FORMATION OF GLYCOGEN IN THE ORGANISM OF THE WHITE RAT AFTER ORAL ADMINISTRATION OF *l*-RHAMNOSE

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(Received for publication, June 9, 1933)

l-Rhamnose, the most common of the naturally occurring methyl pentoses, is found in many glycosides, the carbohydrate constituent of which is solely or chiefly rhamnose. A summary of the discovery, occurrence, and methods of preparation of rhamnose has been presented by Harding (1).

From the physiological point of view, the methyl pentoses are of considerable interest because of their possible relation to the hexoses. Oxidation of the methyl group to a primary alcohol would give rise to a hexose and if such a reaction occurred in the animal organism, the methyl pentoses might serve as sources of glucose and glycogen. *l*-Rhamnose, the naturally occurring optical isomer, by an oxidation of this kind, would be converted to *l*-mannose, the optical antipode of *d*-mannose, the natural form. No well controlled study of the absorption and glycogenic activity of rhamnose has been reported.

In view of these considerations, a study of the behavior of *l*-rhamnose after oral administration to the white rat has been undertaken. Miller and Lewis (2), in reporting a similar investigation with *d*-xylose, have summarized the literature connected with the conversion of the pentoses to glycogen. It should be pointed out that although the older evidence for glycogenesis from pentoses is not convincing, there is to be observed throughout the literature, a tendency to emphasize the superiority of rhamnose over the other common pentoses as a source of glycogen (3, 4). No

experiments, which are concerned with the glycogen deposition after rhamnose feeding, have been reported in which the method introduced by Cori (5) for the study of carbohydrate absorption and glycogenesis in fasting young white rats has been employed. The advantages of this method require no extended comment (2). A study of the influence of phlorhizin on the rate of absorption of rhamnose is also reported.

EXPERIMENTAL

The experimental methods of preliminary treatment of the animals, administration of the carbohydrate, slaughter of the animals, and analyses of the contents of the gastrointestinal tract for sugar and of the livers for glycogen were the same as previously described in the study of xylose (2). Male rats were used in all cases. Rats whose weights after fasting periods of 24 hours were from 100 to 125 gm. were preferred and were employed for most of the experiments.

Since rhamnose differs from both glucose and xylose in its reduction value in the Hagedorn-Jensen method of sugar determination, it was necessary to prepare a table of reduction values in terms of rhamnose. The reduction effected by known amounts of pure rhamnose was determined, the results were plotted, and from this curve, by interpolation, a table of reduction values of rhamnose for use with the Hagedorn-Jensen method was constructed. This table was then checked by a repetition of this procedure.

In one series of experiments, it was desired to determine glucose and rhamnose present together in pure solutions and in the protein-free filtrates obtained from the contents of the gastrointestinal tract. The reduction effected by the mixture was first determined by the Hagedorn-Jensen method. Fermentable sugar was then removed by the yeast treatment recommended by Somogyi (6) and the residual reduction was determined and calculated as rhamnose. The glucose was then calculated by difference, the unequal reduction values of glucose and rhamnose being taken into consideration in the calculation. This method applied to known mixtures of glucose and rhamnose gave satisfactory results.

Rhamnose in the urine was identified by the test with ammonium molybdate in the presence of sulfuric acid (7). In applying this test to rat urine, it was observed that undiluted urine (1 to 2

drops) gave a heavy greenish precipitate even in the absence of rhamnose. However, if the urine were diluted (1:5) and 1 to 2 drops of the diluted urine used for the test, the results were satisfactory. Tests carried out in this manner on normal urines, to which known carbohydrates were added, showed that of the carbohydrates studied (arabinose, xylose, rhamnose, fructose, glucose, and mannose), rhamnose alone gave the characteristic green color.

In the experiments with phlorhizin, the phlorhizin (Merck), recrystallized as suggested by Lusk (8), was injected subcutaneously (50 mg. in 0.5 cc. of olive oil) in the inguinal region for 4 successive days. During this period the animals received the usual stock diet of whole wheat bread, milk, and lettuce. After the period of 4 days of phlorhizin injection, food was withheld for 24 hours and the absorption of the carbohydrate and the glycogen formation were studied in the usual way.

Since the amount of carbohydrate absorbed from the gastrointestinal tract was calculated from the difference between the amount fed and that remaining in the tract, it was necessary to correct the latter value for the reducing power of the tract alone. Cori (5) in studies of carbohydrates which showed high coefficients of absorption considered that this correction was not significant. However, if the absorption coefficient is low, as in the present instance, a considerable error may be introduced if the correction for the normal reduction value of the tract is not made. One group of ten rats of an average weight of 121 gm. served as controls for the determination of the normal reduction values of the contents of the gastrointestinal tract after a 24 hour fast. Values of from 5.5 to 10.5 mg. with an average value of 7.2 mg. (calculated as rhamnose) were obtained. If the residual reductions were calculated as glucose, an average value of 5.6 mg. resulted. These corrections were applied in all the absorption studies.

In a series of check experiments, the accuracy of the experimental procedure was tested as follows. Rats were fed glucose in the usual manner, killed immediately without allowing any time interval for the absorption of the carbohydrate, and the contents of the tract were analyzed. In five experiments, in which glucose (585 to 590 mg.) was fed and the correction for the normal residual reduction was made, 98.4 per cent of the administered

TABLE I

Absorption of l-Rhamnose after Oral Administration to Fasting White Rat and Effect of Injections of Phlorhizin Previous to Rhamnose Feeding upon Rate of Absorption

Rat No.	Weight after 24 hr. fast	Absorption period	Rhamnose fed	Rhamnose absorbed		
				Total	Per 100 gm. rat	Per 100 gm. rat per hr.
	<i>gm.</i>	<i>hrs.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>
49	100	1	268	42	42	42
50	101	1	268	41	40	40
41	104	1	283	44	43	43
38	107	1	550	52	49	49
48	111	1	269	41	37	37
40	113	1	550	62	55	55
43	118	1	283	52	44	44
44	159	1	239	31	19	19
Average..	114			46	41	41
42	110	2	141	47	43	21
107	112	2	264	46	41	20
45	114	2	239	36	31	15
37	115	2	625	52	45	22
105	117	2	321	45	39	19
39	118	2	550	57	48	24
34	145	2	810	52	36	18
127	152	2	310	67	44	22
126	155	2	310	60	32	16
Average..	126			51	40	20
128*	110	2	307	33	30	15
65	118	2	332	71	60	30
47	123	2	269	57	46	23
130	127	2	307	53	42	21
64	132	2	368	59	46	23
129	135	2	307	43	32	16
135	137	2	242	46	34	17
137	143	2	242	40	26	13
134	152	2	242	41	28	14
136	162	2	242	33	20	10
Average..	134			48	36	18

* These rats were given 1 cc. of the rhamnose solution at the beginning of the experiments and another cc. 1 hour later, and were killed 2 hours after the first dose.

TABLE I—*Concluded*

Rat No.	Weight after 24 hr. fast	Absorption period	Rhamnose fed	Rhamnose absorbed		
				Total	Per 100 gm. rat	Per 100 gm. rat per hr.
	<i>gm.</i>	<i>hrs.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>
46	108	3	239	45	41	14
36	113	3	810	51	46	15
32	119	3	281	31	26	8
33	121	3	281	53	44	14
27	144	3	570	47	32	10
66	144	3	338	54	38	12
67	144	3	338	40	28	9
29	155	3	775	55	35	11
Average..	131			47	36	12
79†	110	2	313	27	24	12
58	119	2	368	23	20	10
77	123	2	313	24	20	10
69	127	2	330	20	16	8
85	130	2	443	38	29	14
61	142	2	330	28	20	10
59	171	2	337	15	9	5
Average..	132			25	20	10

† This group received injections of phlorhizin daily for 4 days prior to the fasting period. Details are given in the text.

carbohydrate was recovered. In a further control of the experimental procedure, the absorption of glucose was studied. The absorption coefficients obtained, 239, 209, and 184 for periods of 1, 2, and 3 hours respectively, are comparable to the values of other workers (2, 5, 9-11).

The results of the absorption studies are presented in Table I. The absorption coefficient (mg. absorbed per 100 gm. of rat per hour) decreased as the period allowed for absorption was increased, *i.e.*, 41, 20, and 12 for absorption periods of 1, 2, and 3 hours respectively. These are lower coefficients than those of the common hexoses (5) and of xylose (2, 12). Cori (5) has reported a coefficient of 16 for arabinose over a 2 hour period, a figure comparable to the value obtained for rhamnose during a similar absorption period. Further study of the data of Table I shows that the

absolute amount of rhamnose absorbed per 100 gm. of rat was essentially of the same order of magnitude in all the experiments regardless of the duration of absorption. Thus the absolute values averaged 41, 40, and 36 mg. in 1, 2, and 3 hours respectively. Such results indicate that practically all of the rhamnose which disappeared from the tract must have been absorbed within the 1st hour and that little or no absorption occurred during the subsequent periods. In one series (Table I), the rhamnose was fed in divided doses, an hour apart, and the rats were killed after a total absorption period of 2 hours. Although the absorption per 100 gm. of body weight was slightly high in one experiment (Rat 65), in the other experiments, the values were within the range of the series of the other 2 hour absorption experiments.¹

The fact that the absolute amount of rhamnose absorbed showed no increase over periods of absorption longer than 1 hour suggested that the presence of rhamnose might have resulted in some pathological changes in the intestinal mucosa which hindered further absorption. Magee and Reid (12) have shown that in cats anesthetized with amytal, arabinose and xylose in 0.75 M solution caused immediate and marked inhibition of all movements and shrinkage of the villi.² In order to secure evidence of any toxic action of rhamnose upon the intestinal membrane, rats were fed water, rhamnose, and glucose solutions respectively as in the absorption experiments. After 2 hours, the animals were killed and the upper part of the small intestine was examined microscopically. Dr. Carl V. Weller of the Department of Pathology, to whom we are indebted for the pathological examinations, reported that no morphological changes could be discovered to explain the failure of absorption of rhamnose.

Although no morphological changes were discovered by which the apparent lack of absorption could be explained, the possibility remained that rhamnose might have produced a functional change

¹ Most of the low values were obtained with rats of weights greater than 130 gm. (*e.g.*, Rats 134, 136, 137). We have observed repeatedly that the coefficients of absorption are more consistent if rats of 110 to 130 gm. of body weight are used in the experiments.

² The concentrations of the solutions of rhamnose used in our experiments ranged from approximately 0.7 M (Rats 44 and 45) to 2.4 M (Rats 34 and 36). In every instance except one, 2 cc. of the rhamnose solution were fed. Rat 42 received 1 cc. only.

in the intestinal membrane, which could not be demonstrated microscopically. In order to determine whether the absorptive power of the intestinal mucosa had been altered by the presence of rhamnose in the gut, a group of seven rats was fed a mixture of glucose and rhamnose and the rate of absorption of each sugar was determined after a 2 hour period (Table II). The determination of glucose in the presence of rhamnose has already been described.

TABLE II

Rate of Absorption of Glucose and Rhamnose after Oral Administration of a Mixture of These Two Carbohydrates

Rat No.	Weight after 24 hr. fast		Sugars fed	Sugars absorbed		
				Total	Per 100 gm. rat	Per 100 gm. rat per hr.
	<i>gm.</i>		<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>
86	107	Glucose	645	348	326	163
		Rhamnose	410	69	64	32
87	113	Glucose	587	521	461	230
		Rhamnose	350	53	47	23
91	105	Glucose	562	412	392	196
		Rhamnose	250	47	45	22
92	108	Glucose	562	458	424	212
		Rhamnose	250	47	43	21
104	108	Glucose	593	375	347	173
		Rhamnose	312	75	70	35
109	119	Glucose	670	443	373	186
		Rhamnose	262	65	55	27
110	116	Glucose	670	408	352	176
		Rhamnose	262	65	56	28
Average..	111	Glucose		423	382	191
		Rhamnose		60	54	27

In control experiments in which glucose was fed alone, absorption coefficients ranging from 161 to 269 with an average of 209 were obtained. When glucose was fed with rhamnose, the absorption coefficients of glucose ranged from 163 to 230 with an average of 191. This indicates that the ability of the intestinal membrane to absorb glucose was not significantly altered by the presence of rhamnose.³ Cori (13), in experiments in which a mixture of equal

³ Further evidence that the glucose was absorbed and metabolized normally when introduced into the gastrointestinal canal with rhamnose is

amounts of glucose and galactose was fed, observed that the rate of absorption of both these readily absorbed sugars was reduced to such an extent, that the total amount of sugar absorbed was not greater than if either sugar alone were presented for absorption. Since rhamnose is so poorly absorbed, its presence would not be likely to alter significantly the rate of absorption of the glucose. These experiments and the pathological examination of the intestine seem to indicate that rhamnose did not produce any toxic effect which changed the absorptive power of the intestinal mucosa of the rat.

McCance and Madders (14) made a comparative study of the rates of absorption of xylose, rhamnose, and arabinose in the white rat by the Cori method. They concluded that rhamnose was poorly absorbed. Inasmuch as male rats of 230 to 300 gm. were used and the weights of the individual animals used are not stated, it is impossible to make any more direct comparison of their results with our own. Four rats only were used in experiments of 3 hours duration.

It has been reported that under the influence of phlorhizin, the rate of absorption from the gut is altered. Wilson (11) in experiments in which the Cori procedure was used, showed that the rate of absorption of glucose in phlorhizinized rats was only 70 per cent of that of normal animals. It has been shown (12, 15) that glucose absorption in the white rat was increased by the presence of phosphates in the intestine, while the rate of absorption of the pentoses (xylose) was not altered. It was considered of interest to study the effect of phlorhizin on the absorption of rhamnose in order to determine whether the effect of phlorhizin on the absorption of a pentose varied from the effects of this glycoside on absorption of glucose. The results are presented in the last series of experiments of Table I. When rhamnose was fed to phlorhizinized rats, both the coefficient of absorption and the absolute amount of rhamnose absorbed were decreased to about 50 per cent

afforded by analyses of the glycogen content of the liver made on these same animals. The per cent of liver glycogen after the 2 hour absorption period ranged from 0.94 to 2.02 per cent with an average of 1.40 per cent. In control experiments in which glucose was administered alone, the average per cent of liver glycogen after a 2 hour absorption period was 1.43.

of the normal value. The absorption of rhamnose and that of glucose are affected similarly by phlorhizin injections.

The glycogen content of the liver and in some animals that of the rest of the body were determined in the experiments detailed in Table I. In no case was the glycogen content found to be increased above the fasting level after the oral administration of rhamnose. This is presumably to be ascribed in part to the slow absorption and rapid excretion of the rhamnose after administration, but is undoubtedly related also to the difficulty in metabolizing the rhamnose. The urine of rats fed rhamnose reduced Benedict's alkaline copper solution and gave strongly positive tests for rhamnose even during the 1st hour of absorption. These observations are in harmony with those of former workers and indicate that absorbed rhamnose was rapidly excreted by the kidneys as a foreign substance.

A similar rapid excretion of pentose has been reported by Grafe (16) in experiments with dogs and men who observed that 40 to 50 per cent of the xylose fed was excreted by the kidneys. He obtained no evidence of glycogen formation after oral administration of xylose to dogs, a finding supported by recent studies of Magendantz (17) with dogs. Grafe, unable to show any difference in the utilization of xylose in normal and diabetic men, concluded that, "Daher bestehen für seine Verwendung beim Zuckerkranken als Zuckerersatz keine Bedenken."

It is of interest to compare the behavior of rhamnose with that of the other pentose, *d*-xylose, recently studied in this laboratory (2). The absorption of rhamnose in the initial period of absorption was greater than that of xylose. With one exception (Rat 44, Table I), the amount absorbed in 1 hour was greater than the highest value (34 mg.) obtained with xylose, while the average rhamnose absorption (41 mg.) during this period was definitely higher than the average obtained for xylose (29 mg.). The absorption of xylose, however, continued at a slightly greater rate during subsequent periods (2 and 3 hours) while the absorption of rhamnose occurred to a very limited extent, if at all, after the 1st hour. It is impossible for us to explain this difference in behavior in the light of our present information. We do not consider that it is to be ascribed to any toxic substance present in the rhamnose used since absorption of glucose was shown to proceed normally in the

presence of the rhamnose. No evidence is afforded by these studies to indicate any superior utilization of rhamnose as compared with xylose (2).

SUMMARY

1. The absorption coefficients of *l*-rhamnose, the methyl pentose of most common occurrence in nature, from the gastrointestinal tract of the white rat, as determined by the method of Cori (5), decreased with the increased duration of the period of absorption; *i.e.*, absorption coefficients (mg. per 100 gm. of rat per hour) of 41, 20, and 12 for 1, 2, and 3 hour absorption periods respectively. The absolute amount of rhamnose absorbed per 100 gm. of rat was nearly constant in all the periods of absorption regardless of the duration of absorption, 41, 40, and 36 mg. respectively. This suggests that no significant absorption of rhamnose occurred after the 1st hour.

2. After oral administration of glucose in mixture with rhamnose, glucose was as well absorbed from the gastrointestinal canal as when glucose was fed alone. This is believed to indicate that the poor absorption of rhamnose is not to be ascribed to any toxic effects of the rhamnose fed.

3. In phlorhizinized rats, the rate of absorption of rhamnose was decreased to about 50 per cent of the normal value.

4. No evidence was obtained that under the experimental conditions, oral administration of rhamnose resulted in a deposition of glycogen in the liver or that rhamnose was superior to xylose as a precursor of glycogen.

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WAVERLY PRESS, INC
BALTIMORE, U. S. A.